Evaluation of native antagonists for the management of stem rots of cluster bean caused by *Sclerotium rolfsii* Sacc.

Muthukumar, A[1]., Suthin Raj, T[2]., Sudha, A[3] and Udhayakumar, R[2]

ABSTRACT

Cluster bean, *Cyamopsis tetragonoloba* (L.) Taub is an important annual legume crop of *kharif* season in arid and semi-arid regions of the Indian subcontinent. Six fungal antagonists and four bacterial antagonists were evaluated against *Sclerotium rolfsii in vitro* and *in vivo* condition to develop an effective management strategy for stem rot of cluster bean. During *in vitro* studies, native fungal and bacterial antagonists were inhibited the mycelial growth of pathogen. Among the fungal antagonists tested, the isolate-I_4 (THM) showed the maximum growth inhibition of *S. rolfsii* up to 68.10 per cent. The bacterial antagonist Isolate-I_3 (PFV) recorded the maximum inhibition zone of 13.33 mm and a minimum of 23.00 mm mycelial growth of *S. rolfsii*. Pot culture studies revealed that the combined application of T_9-*T. harzianum* (THM) + *P. fluorescens* (PFV) (ST+ SA) through seed treatment and soil application recorded the minimum incidence of stem rot (4.66 per cent) and maximum plant height, number of pods/plant and pod length.The findings reported in the present study supported the applicability of isolate-I_4 (THM) Isolate-I_3 (PFV) as a possible alternative to fungicides for the control of root rot in black gram.

Keywords: *Cyamopsis tetragonaloba*, *Trichoderma*, *Pseudomonas*, Plant growth promotion

MS History: 22.08.2022(Received)-30.10.2022(Revised)- 10.11.2022 (Accepted)

Citation: Muthukumar, A., Suthin Raj, T., Sudha, A and Udayakumar, R. 2022. Evaluation of native antagonists for the management of stem rots of cluster bean caused by *Sclerotium rolfsii* Sacc. *Journal of Biopesticides*, **15**(2): 162-172.

DOI: 10.57182/jbiopestic.15.2.162-172

INTRODUCTION

Cluster bean *Cyamopsis tetragonaloba* (L.) Taub is commonly known as guar means 'cow food' belonging to the family leguminaceae. It is an annual arid and semi-arid legume crop (Singh *et al*., 2001) grown as green manure, as forage crop for cattle and as a vegetable crop for human consumption. The crop has got a special importance because of gum content in its seed. India earns around 80 crore rupees in foreign exchange annually by export of cluster bean gum. Cluster bean is a rich source of high quality galactomannan gum and protein rich (40-50%) guar meal as animal feed. Seed gum is used in various industries such as textiles, paper, cosmetics, explosives and food processing. Besides the gum preparation, cluster bean is emerging as a potential source of vegetable protein for human beings. The green and tender pods of guar are cooked as favourite vegetables in many parts of the country including South India (Choudhary and Sindhu, 2015). India is the largest producer of Guar with 80% among the world production, followed by Pakistan with 10-15%. Cluster bean is a native to the Indian subcontinent. It is an erect, bushy, annual herbaceous legume up to 3 m height with trifoliate leaves up to 10 cm long and white flowers. The pods are straight, hairy, pale shiny green, up to 12 cm long and contain 5 to 12 hard seeds. The area under cluster bean production in India is 4.26 million ha with a production of 2.42 million tones and productivity of 567 kg/ha (Anonymous, 2020). Rajasthan is the biggest cluster bean producer state contributes

about 80 per cent of the total cluster bean production in the country. In Rajasthan, area under the cluster bean crop is 35.30 lakh hectare with production of 14.04 lakh tonnes and productivity 398 kg/ha (Anonymous, 2020).

The fungal infection causes variations in protein contents of plant parts. Being a multipurpose crop, there is a great demand of organic cluster bean. Sustainable cluster bean cultivation is continuously challenged by diseases that cause quantitative and qualitative losses in yield. The cluster bean plants infected with several fungal diseases, of which stem rot caused by *Sclerotium rolfsii* (Ronakkumar and Sumanbhai, 2014) is a soil borne disease which causes considerable damage to the crop and yield loss was estimated up to 50-70 per cent under field condition (Ronakkumar and Sumanbhai, 2014). The pathogen produces sclerotia which over winter in soil and on plant debris and can survive for a long period causing disease in the following season (Punja, 1985) Thus, the control of the disease is very difficult by conventional means (Punja, 1998; Sarma *et al.,* 2000). The first attempt to control the disease is by using chemical means (Ganeshan, 1997). Although fungicides have shown promising results in controlling the fungal diseases, phytotoxicity and fungicide residues are major problems leading to environmental pollution and human health hazards. Sanitation using sterile (or) clean water supplies, application of organic compost and regulation of watering and temperature contributed to the management of the disease to some extent. Thus, existing control measures are not effective for the control of stem rot disease. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and eco-friendly method for plant disease management. Soil has enormous untapped potential antagonistic microbes *viz., Trichoderma* spp. *Bacillus* spp. and fluorescent pseudomonads which show antagonistic effects against soil borne plant pathogens. The use of biocontrol agents is gaining importance for their plant growth promotion and disease reduction abilities (Nagamma and Nagaraja, 2015; Dwivedi and Prasad, 2016; Dhivya, 2020). The successful application of antagonistic microorganisms for the control of *S. rolfsii* has been reported by several workers in various crops (Muthukumar and Venkatesh *et al*., 2014; Ekundayo *et al*., 2015; Pacheco *et al*., 2016). With this background, the present study has been undertaken with the following objectives. To evaluate the antagonistic potential of native bio-agents and to assess the efficacy of antagonistic agents in managing stem rot disease under pot culture conditions.

MATERIALS AND METHODS

Cluster bean seeds var. *Pusa Mausami* purchased from Department of Horticulture, TNAU, Coimbatore were used in the pot culture studies.

Isolation and maintenance of pathogen

The stem rot symptoms were collected from major cluster bean growing tracts of Tamil Nadu pertaining to districts such as Cuddalore, Dindigul, Erode, Namakkal and Salem. The infected plant materials brought back from the field were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface sterilized in 0.5% sodium hypochlorite solution for 5 min. and rinsed in three changes of sterile distilled water. The segments were separately dried in between sheets of sterile filter paper and placed (3 pieces per plate) on fresh potato dextrose agar (PDA) medium (Ainsworth, 1961) impregnated with streptomycin, and incubated for seven days at 28±2°C.

A total of seven isolates (I_1 to I_7) causing stem rot was isolated from infected plant samples collected from different tracts of Tamil Nadu. The fungal growth on 5^{th} day, which arose through the sclerotial bodies was cut by inoculation loop and transferred aseptically to the PDA slants and allowed to grow at room (28±2°C) temperature to obtain the pure culture of the fungus. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically. The purified isolates were identified as *S. rolfsii* based on morphological and colony characteristics (Punja and Damini, 1996; Sarma *et al*., 2002; Watanabe, 2002b). Based on pathogenicity studies

the highly virulent isolate of *S. rolfsii*-(I_1) was used for further studies.

Isolation of native antagonists from cluster bean rhizosphere

Antagonistic fungi (six locations) and bacteria (four locations) were isolated from the rhizosphere soil of different cluster bean growing tracts of Tamil Nadu. The plants were pulled out gently with intact roots and the excess soil adhering on roots was removed gently. one gram of rhizosphere soil was transferred in to 10 mL test tube containing 10 mL of sterile distilled water. After thorough shaking, the antagonist in the suspension was isolated by serial dilution plate method. From the final dilutions of 10^{-4} 10^{-6} and 10^{-7} one mL of each aliquot was pipetted out, poured in to sterilized Petri plate containing *Trichoderma* selective medium (Elad and Chet, 1983) (TSM), King's B medium (King *et al.*, 1954), nutrient agar medium separately and they were gently rotated clockwise and anti-clockwise for uniform distribution and incubated at room temperature (28 ± 2^0C) for 7 days for fungi and 48 hrs for bacteria. Colonies with characteristics of *Bacillus* spp., *Pseudomonas* spp. were isolated individually and purified by streak plate method (Rangaswami, 1993) on nutrient agar medium and King's B medium, respectively. *Trichoderma* spp. was isolated from TSM medium and the culture purified on PDA medium. The pure cultures were maintained on the respective agar slants at 4^oC. The *Trichoderma* spp. were identified based on the key characters proposed by Rahman *et al*. (2011); Muthukumar *et al*. (2011). *Pseudomonas* and *Bacilus* spp. were identified based on biochemical test (Holt *et al*., 1994).

The identified fungal isolates were designated as THO - *T. harzianum* (Ottanchatram) ; TVK- *T. viride* (Kalipatti); THK- *T. harzianum* (Kavarapattu); THM- *T. harzianum* (Melamoongiladi) TVV - *T. viride* (Vattur) and THKO - *T. harzianum* (Koothanatham). For *in vitro* studies comparison, *T. harzianum* obtained from Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore was designated as THCB. The identified bacterial isolates were designated as BSK - *B. subtilis* (Kavarapattu); PFC- *P. fluorescens* (Ottanchatram); PFV -*P. fluorescens* (Vattur) PFM- *P. fluorescens* (Melamoongiladi). For *in vitro* studies comparison, *P. fluorescens* was obtained from Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore was designated as PFCB.

***In vitro* testing of fungal antagonists**

The antagonistic activity of fungal antagonists against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971) using PDA medium. At one end of the sterile Petri plate containing fifteen mL of sterilized and solidified PDA medium, a six mm mycelial disc obtained from five days old culture of *Trichoderma* species was placed under aseptic conditions. Similarly at the opposite end approximately 75 mm away from the *Trichoderma* culture disc, a six mm culture disc of pathogen obtained from seven days old culture of *S. rolfsii* was placed and incubated. A control was maintained by inoculating *S. rolfsii* alone at one end of the Petri plate. The plates were incubated at room temperature (28±2°C) for seven days. The per cent inhibition of mycelial growth was calculated according to Vincent (1929). Based on the dual culture technique the effective *Trichoderma* species were identified and used for further studies.

***In vitro* testing of bacterial antagonists**

The antagonistic activity of five bacterial antagonists against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971) using PDA medium. At one end of the sterile Petri plate containing fifteen mL of sterilized and solidified PDA medium, a six mm culture disc of pathogen obtained from seven days old culture of *S. rolfsii* was placed at 1.5 cm away from the margin of the Petri plate. Similarly, one cm long streak was gently made onto the medium using 48 hrs old culture of bacterial isolates just opposite to the pathogenic culture at equidistance under aseptic condition. A control was maintained by inoculating *S. rolfsii* alone at one end of the Petri plate. The plates were incubated at room

temperature (28±2°C) for 48 hrs. The radial growth (in mm) of the pathogen was measured after incubation. The effective antagonists were selected based on the inhibition of the growth of pathogen. The per cent inhibition of mycelial growth was calculated according to Vincent (1929).

Seed treatment and soil application with antagonists

An experiment was conducted at Department of Plant Pathology, Faculty of Agriculture, Annamalai University during the year 2019-2020 under pot culture condition. A pot culture experiment was laid out by using three effective bio-control agents *viz.*, *T. harzianum* (THM), *P. fluorescens* (PFV) and Bavistin for chemical comparison. Each treatment was replicated three times in Randomized block design. Sterilized soil (1.0 kg) was mixed with the pathogen inoculum @ 5 grains (multiplied on sorghum grains) and filled in 15 x 30 cm diameter earthen pots. The cluster bean seeds were treated with talc based formulation of the antagonists (3 seeds of cluster bean var. Pusa Mausami/pot) were sown and after thinning one plant was maintained and fertilizer dose applied as recommended. Equal quantity of bio formulation (w/w) were mixed and from the final mixture @2.5kg/ha was taken for soil application. Talc based formulations of the antagonists were applied to the soil at 20 days before sowing. Bavistin was applied as soil drenching at three days before sowing. Cluster bean seeds treated with Bavistin @ 2g/kg as chemical comparison. Pathogen alone inoculated pots served as control. Inoculated plants were kept in open place for observation and the pots were irrigated as when required. The observation on the incidence of collar rot was recorded at 35 days after sowing. The number of plants showing typical symptoms due to *S. rolfsii* was observed and Per cent disease incidence was calculated using formula (Kokalis-Burelle *et al.*, 1997). The treatment schedule followed is T_1[*T. harzianum* (THM) ST @ 6g/kg of seed], T_2[*T. harzianum* (THM) SA @2.5kg/ha], T_3 [T1+T2], T_4 [*P. fluorescens* (PFV) ST @12 g/kg of seed], T_5 [*P. fluorescens* (PFV) SA @2.5kg/ha], T_6 [T4+T5], T_7 [*T. harzianum* (THM) ST +*P. fluorescens*(PFV) ST], T_8 [*T. harzianum* (THM) SA +*P. fluorescens* (PFV) SA], T_9 *T. harzianum* (THM) + *P. fluorescens*(PFV) (ST+ SA)], T_{10} [Bavistin ST@ 2 g/kg of seed+ SD @0.2%] and T_{11} [Inoculated control](ST-Seed Treatment; SA-Soil Application; SD-Soil Drenching)

Statistical analysis

The data on the effect of the treatments on the growth of pathogen and disease incidence were analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT). The data on disease incidence was arcsine transformed before undergoing statistical analysis (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT version 92-1eveloped by the Biometrics Unit of the International Rice Research Institute, The Philippines.

RESULTS

***In vitro* inhibition of *Trichoderma* species against *S. rolfsii* (I_1)**

All the native isolates of *Trichoderma* species inhibited the mycelial growth of *S. rolfsii* (Table 1). Among the native *Trichoderma* isolates tested, the isolate-I_4 (THM) showed the maximum growth inhibition of *S. rolfsii* up to 68.10 per cent and it was on par with I_7 (THCB). This was followed by the isolate-I_2 (TVK) and I_5 (TVV) in the decreasing order of merit. The least growth inhibition of pathogen (54.70 per cent) was exhibited by the isolate-I_6 (THKO).

***In vitro* inhibition of mycelial growth of *S. rolfsii* (I_1) by native bacterial isolates**

The results presented in the table 2 revealed varying degree of antagonism by the bacterial isolates against *S. rolfsii*. Among the isolates tested, isolate-I_3 (PFV) recorded the maximum inhibition zone of 13.33 mm and a minimum of 23.00 mm mycelial growth of *S. rolfsii* accounting for 74.25 per cent reduction in the mycelial growth over control and it was on par with I_5 (PFCB). This was followed by isolate-I_2 (PFO) (26.00 mm).

Table 1. *In vitro* antagonism of *Trichoderma* species against S. *rolfsii* (I_1)

SI. No.	Location	Antagonist	Mycelial growth (mm)	Per cent inhibition over control
I_1	Ottanchatram	THO	37.00[c]	58.42
I_2	Kalipatti	TVK	35.00[b]	60.70
I_3	Kavarapattu	THK	39.00[d]	56.17
I_4	Melamoongiladi	THM	28.33[a]	68.10
I_5	Vattur	TVV	35.33[b]	60.30
I_6	Koothanatham	THKO	40.33d[e]	54.70
I_7	DPP	THCB	28.66[a]	67.80
-	Control	-	89.00[f]	-

Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

Table 2. *In vitro* inhibition of mycelial growth of *S. rolfsii* (I_1) by native bacterial isolates

I. No.	Isolates	Mycelial growth (mm)	Per cent inhibition over control	Inhibition zone (mm)
I_1	BSK	30.66[c]	55.00	9.66[c]
I_2	PFO	26.00[b]	60.22	11.0[b]
I_3	PFV	23.00[a]	63.58	13.33[a]
I_4	PFM	29.66[c]	56.12	9.66[c]
I_5	PFCB	23.66[a]	62.84	13.00[a]
-	Control	89.33[d]	-	-

Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

The least mycelial growth inhibition was observed with the isolate-I_1.

Seed treatment and soil application with antagonist on plant growth

Studies on the effect of antagonist under pot culture condition revealed that the combined application of T_9-*T. harzianum* (THM) + *P. fluorescens* (PFV) (ST+ SA) recorded the minimum incidence of stem rot). This was on par with the chemical treatment which recorded an incidence of 4.33%. These were followed by (T_8-*T. harzianum* (THM) SA+ *P. fluorescens* (PFV) SA) which recording 6.33% stem rot incidence (Plate 13). The maximum disease incidence was recorded with inoculated control (Table 3).

Plant growth and production

Generally all the treatments with bioformulations showed increased plant height and yield when compared to control (Table 4). Of these, the treatment consisting of T_9-*T. harzianum* (THM)+ *P. fluorescens* (PFV) (ST+ SA) recorded maximum plant height, more number of pods/plant and maximum pod length at 120 days after sowing. This was on par with the treatment T10 - Bavistin which recorded a plant height of 110.00 cm with more number of pods/plant and maximum pod length (9.83 cm). It was followed by the treatment T_8- *T. harzianum* (THM) SA+ *P. fluorescens* (PFV) SA (90.00 cm; 48.00; 8.66 cm)). In general all the treatments except control recorded lowest plant height, less number of pods/plant and minimum pod length of 2.33 cm.

Number of seeds/pod and pod yiled

In general maximum number of seeds/pod (12/pod) and highest pod yield (8.0 t/ha) which received through the treatment T_9-*T. harzianum* (THM) + *P. fluorescens* (PFV) (ST+ SA) followed by T_8. Less number of seeds/pod (3.0) and lowest pod yield was observed in control (2.50 t/ha).

Table 3. Effect of seed treatment and soil application with consortium of antagonists on the incidence of stem rot of cluster bean

Treatments	Disease incidence (%)
T_1-*T. harzianum* ST	12.00^{e} (20.26)
T_2-*T. harzianum* SA	12.66^{ef} (20.84)
T_3-*T. harzianum* ST+SA	10.66^{e} (19.05)
T_4-*P. fluorescens* ST	11.00^{e} (19.36)
T_5-*P. fluorescens* SA	10.33^{e} (18.74)
T_6-*P. fluorescens* ST+ SA	9.00^{d} (17.45)
T_7-*T. harzianum* ST+ *P. fluorescens* ST	7.66^{c} (16.06)
T_8-*T. harzianum* SA+ *P. fluorescens* SA	6.33^{b} (14.57)
T_9-*T. harzianum* + *P. fluorescens* (ST+ SA)	4.66^{a} (12.46)
T_{10}- Bavistin ST@ 2 g/kg of seed+ SD @0.2%	4.33^{a} (12.01)
T_{11}-Inoculated control	67.66^{g} (55.34)

ST-Seed Treatment; SA-Soil application; SD-Soil drenching; Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

Table 4. Effect of seed treatment and soil application with antagonists on plant growth parameters and yield of cluster bean

Treatments	Plant height (cm)	No. of pods/plant	Pod length (cm)	No. of seeds/pod	Yield/plant (g)	Yield/ha (t)
T_1-*T. harzianum* ST	71.66^{h}	33.33^{i}	7.16^{bc}	7^{d}	145.66^{h}	7.26^{b}
T_2-*T. harzianum* SA	73.33^{g}	35.00^{h}	7.26^{b}	7^{d}	145.33^{h}	7.25^{b}
T_3-*T. harzianum* ST+SA	75.33^{f}	37.33^{g}	7.33^{b}	8^{c}	147.00^{g}	7.28^{b}
T_4-*P. fluorescens* ST	77.33^{e}	39.00^{f}	7.20^{b}	8^{c}	149.00^{f}	7.35^{b}
T_5-*P. fluorescens* SA	78.00^{e}	41.66^{e}	7.43^{b}	8^{c}	152.00^{e}	7.35^{b}
T_6-*P. fluorescens* ST+ SA	80.66^{d}	43.00^{d}	7.66^{b}	8^{c}	158.33^{d}	7.40^{b}
T_7-*T. harzianum* ST+ *P. fluorescens* ST	88.33^{c}	46.33^{c}	8.33^{b}	10^{b}	170.00^{c}	7.60^{b}
T_8-*T. harzianum* SA+ *P. fluorescens* SA	90.00^{b}	48.00^{b}	8.66^{b}	10^{b}	180.33^{b}	7.72^{b}
T_9-*T. harzianum* + *P. fluorescens* (ST+ SA)	110.33^{a}	52.33^{a}	9.80^{a}	12^{a}	200.66^{a}	8.00^{a}
T_{10}- Bavistin ST@ 2 g/kg of seed+ SD @0.2%	110.00^{a}	52.00^{a}	9.83^{a}	12^{a}	200.00^{a}	8.10^{a}
T_{11}-Inoculated control	32.66^{i}	17.00^{j}	2.33^{d}	3^{e}	60.66^{i}	2.50^{c}

ST-Seed Treatment; SA-Soil application; SD-Soil drenching; Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05).

DISCUSSION

In vitro antagonism

All the native *Trichoderma* species reduced the mycelial growth of *S. rolfsii*. Among the isolates tested, the isolate-I_4 (THM) showed the maximum growth inhibition of *S. rolfsii*. Similarly, Sab *et al*. (2014) reported that *T. harzianum*-55 IIHR recorded maximum inhibition of 70% followed by *T. harzianum* NBAII with 63% growth inhibition of *S. rolfsii*. Patel and Rakholiya (2016) reported that *T. harzianum* showed maximum growth inhibition of 54.72% after 7 days of incubation and appeared to be the most superior over all other bio-agents tested followed by *T. viride* (50.42%). Manandhar *et al*. (2019) reported that a total of ten *Trichoderma* isolates was examined for *in vitro* antagonism against *S. sclerotiorum*. Among them, the isolate T363 was found to exhibit more than 80 per cent inhibition of *S. sclerotiorum.* The results are in agreement with earlier workers (Darvin *et*

al., 2013; Bhat *et al.,* 2015; Siddique *et al.,* 2016). *T. harzianum* was found to inhibit *in vitro* growth of *S. rolfsii* by coiling around mycelium of *S. rolfsii* resulting in lysis of hyphae (Fouzia and Seleem Shahazad, 2005). Antagonists may also affect the growth of pathogen either through antibiosis (or) mycoparasitism. Besides they may also produce antifungal phenolic compounds (viridin, gliotoxin and trichodermin) (Sababanday *et al.,* 2008; Rahel Ratnakumari *et al.,* 2011) which might be responsible for the inhibition of pathogen.

***In vitro* inhibition of mycelial growth**

All the five bacterial isolates showed varying degrees of antagonism to *S. rolfsii*. Among the isolates tested, isolate-I_3 (PFV) recorded the maximum inhibition zone of 13.33 mm and a minimum of 23.00 mm mycelial growth of *S. rolfsii.* Similar observations on variation in antagonistic efficacy between isolates were recorded by several workers. Similarly, Narayana Bhat *et al.* (2015) reported that the bacterial antagonist *P. fluorescence* pf3 gave highest inhibition zone of *S. rolfsii* (10.5 mm) and was significantly superior to other isolates. Koopa and Krishnaraj (2017) reported that the highest mycelial growth inhibition of *S. rolfsii* by *Pseudomonas* isolate AUDP 48 under dual culture technique. Paramasivan *et al.* (2019) reported that among the six isolates of *Pseudomonas* sp. 22 tested for their antagonistic activity against *S. rolfsii* by dual culture *P. fluorescens* (SBHRP2) significantly exerted highest per cent mycelial growth inhibition of 66.36 followed by *P. fluorescens* (SBHRPF4) and *P. chlororaphis* (PA 23) recorded 65.27 and 64.77 per cent mycelial growth inhibition over the control. Production of siderophores and chitinases are two factors that may be involved in biological control activity. Indeed, it is known that chitinolytic activity and siderophore production are correlated with antifungal activity (Castoria *et al.,* 2001; Kamensky *et al.,* 2003; Quecine *et al.,* 2008). In addition, *P. fluorescens* is capable of solubilizing phosphate and producing IAA, characteristics that may enhance its potential use as an effective biological control agent to contribute to the control of *S. rolfsii*. Mahesh (2007) suggested that fungal growth is mainly inhibited by HCN production and siderophore production. In addition to this, *Pseudomonas* spp. are well known for production of broad spectrum antibiotics such as phenazine-1-carboxylic acid (PCA), 2, 4-diacetylphloroguoinol (2,4-DAPG), Pyoluteorin, Pyrrolnitrin and antibiosis which proved to be a major mechanism involved in their biocontrol activity (O' Sullivan and O'Gara, 1992). All these earlier results lend support to the present findings.

Seed treatment and soil application

The results of the present study clearly indicated that the combined application of T_9-*T. harzianum* (THM) + *P. fluorescens* (PFV) (ST+ SA) recorded the minimum incidence of stem rot with maximum plant height and yield of cluster bean. Similar observations were made by Muthukumar and Venkatesh (2014) reported that the combined application of *T. harzianum* + *P. fluorescens* through seedling dip treatment recorded minimum incidence of collar rot of peppermint caused by *S. rolfsii*. The combined application of antagonistic bacterial formulations EPI (Pf-5) +KGI (Bs-4) +KPI (Pf-7) recorded minimum wilt incidence and increased plant height, dry weight and fruit yield of tomato when compared to control (Sundaramoorthy and Balabaskar, 2013). Seed treatment with *Trichoderma* strains (*T. harzianum* TR05 + *T. asperellum* TR08) recorded maximum germination percentage with minimum incidence of collar rot of tomato caused by *S. rolfsii* (Islam *et al*., 2017). Shiva Kant Kushwaha *et al.* (2018) reported that seed treatment with native bacterial isolate-Iso-32 (10^9cfu/mL) recorded minimum brinjal collar rot incidence of 6.3 per cent and exhibit 88.5 per cent reduction over control. The antagonistic bioinoculants (*T. harzianum* and *P. fluorescens*) applied through soil has resulted in quicker proliferation leading to faster establishment of the antagonistic bio inoculants in

the rhizosphere. The observations on the establishment of bio-control agents were typically present at higher concentration at 20 days after application. This might be the reason for the increased activity of these antagonists as later stages of crop growth. In addition to this by their high rhizosphere competence leading to improved root health might have protected cluster bean seedlings against stem rot disease. Such type of faster establishment has been reported by Weller (1988); Ramanathan (1989) and Cliquet and Scheffer (1996). The increase in plant growth might be due to the growth-promoting compounds such as auxins, gibberellins and cytokinins produced by antagonistic microorganisms (Pal *et al.*, 2000; Gholami *et al.*, 2009; Son *et al*., 2014).

Combined application of antagonists (T_9-*T. harzianum* (THM) + *P. fluorescens* (PFV- ST+ SA) through seed and soil application increased rhizosphere competence of the antagonists besides activating induced systemic resistance and have led to the suppression of pathogen resulting in the reduced incidence of the disease. The combined application of bio-control agents provides an alternative to chemical fungicides by offering environmentally safer management of stem rot in cluster bean.

REFERENCES

Ainsworth, G. C. 1961. Dictionary of fungi. Common Wealth Mycological Institute, Kew Burrey, England, **pp**. 547.

Anonymous, 2020. Directorate of Economics and Statistics, Government of Rajasthan, Jai

Bhat, N. M., Sardana, H. R., Dinesh Singh, Chitra Srivastava and Mobin Ahmad. 2015. Evaluation of chemicals and bioagents against *Sclerotium rolfsii* causing southern blight of bell pepper (*Capsicum annuum*). *Indian Phytopathology*, **68**(1): 97-100.

Castoria, R., De Curtis, F., Lima, G., Caputo, L., Pacifico, S. and De Cicco, V. 2001. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: Study on its modes of action. *Post-Harvest Biology and Technology*, **22**: 7-17.

Choudhary, S. M. and Sindhu, S. S. 2015. Suppression of *Rhizoctonia solani* root rot disease of cluster bean (*Cyamopsis tetragonoloba*) and plant growth promotion by rhizosphere bacteria. *Plant Pathology Journal*, **14**(2): 48-57.

Cliquet, S. and Scheffer, R. F. 1996. Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* using *Trichoderma* spp. applied as industrial film coating on seeds. *European Journal of Plant Pathology*, **102**: 247-255.

Darvin G, Venkatesh, I. and Reddy, G.N. 2013. Evaluation of *Trichoderma* spp. against *Sclerotium rolfsii in vitro*. *International Journal of Applied Biology and Pharmaceuticlal Technology*, **4**(4): 268-272.

Dennis, L. and Webster, J. 1971. Antagonistic properties of species group of *Trichoderma* I. Production of non-volatile antibiotics. *Transactions of British Mycological Society*, **57**: 25-39.

Dhivya, A. 2020. Studies on the biological management of foot rot of brinjal (*Solanum melongena* L.) caused by *Sclerotium rolfsii* Sacc. *M.Sc (Ag.), Thesis*, Faculty of Agriculture, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India, **pp**. 224.

Dwivedi, S. K. and Ganesh Prasad. 2016. Integrated management of *Sclerotium rolfsii*. *European Journal of Biomedical and Pharmaceutical Sciences*, **3**(11): 137-146.

Ekundayo, E. A., Ekundayo, F. O. and Osinowo, I. A. 2015. Antifungal activities of *Trichoderma viride* and two fungicides in controlling diseases caused by *Sclerotium rolfsii* in tomato plants. *Advances in Applied Science Research*, **6**(3): 12-19.

Elad, Y., Chet, I., Boyle, P. and Henis, Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* by scanning electron microscopy and fluorescence microscopy. *Phytopathology*, **73**: 253-258.

Fouzia, Y., Qub, and Seleem, S. 2005. Pathogenicity of *Sclerotium rolfsii* on different crops and effects of inoculum density on colonization of mung bean and sunflower roots. *Pakistan Journal of Botany,* **37**: 175-780.

Ganeshan, G. 1997. Fungicidal control of *Sclerotium* basal rot of cluster bean cv. Pusa Naubahar. *Indian Phytopathology*, **50**(4): 508-512.

Gholami, A., Shahsavani, S. and Nezarat, S. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *International Journal of Biology and Life Sciences*, **1**: 35-40.

Gomez, K. A. and Gomez, A. A. 1984. Statistical Procedure for Agricultural Research. John Wiley and Sons, New York.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, T. 1994. Bergey's Manual of Determinative Bacteriology, 9th edn. Baltimore : Williams & Wilkins.

Islam, M., Hossain, D. M., Nonaka, M. and Harada. 2017. Biological control of tomato collar rot induced by *Sclerotium rolfsii* using *Trichoderma* species isolates in Bangladesh. *Archives of Phytopathology and Plant Protection,* **50**: 109-116.

Kamensky, M., Ovadis, M., Chet, I and Chernin, L. 2003. Soil borne strain IC 14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia Sclerotium* diseases. *Soil Biology and Biochemistry,* **35**: 323-331.

King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanine and fluorescein. *Journal of Laboratory and Clinical Medicine,* **44**: 301-307.

Kokalis-Burelle, N., Porter, D. M., Rodriquez-Kabana, R., Smith, D. H and Subrahmanyam P, 1997. Compendium of peanut disease (2nd edn). *APS Press*, St. Paul.

Koopa, K. P. and Krishnaraj, P. U. 2017. Effect of Actinobacteria and *Pseudomonas* spp. against *Sclerotium rolfsii* in groundnut. *International Journal of Current Microbiology and Applied Sciences,* **6**(10): 229-245.

Mahesh, G. M. 2007. Growth promotion and disease suppression ability of *Pseudomonas fluorescens* on acid lime. M.Sc Agri Thesis, Akola.

Manandhar, S., Pant, B., Manandhar, C and Baidy, S. 2019. *In-vitro* Evaluation of bio-control agents against soil borne plant pathogens. *Journal of Nepal Agricultural Research Council,* **5**: 68-72.

Muthukumar, A. and Venkatesh, A. 2014. Biological induction of systemic resistance to collar rot of pepper mint caused by *Sclerotium rolfsii*. *Acta Physiologiae Plantarum,* **36**: 1421-1431.

Muthukumar, A., Eswaran, A. and Sanjeevkumar, K. 2011. Exploitation of *Trichoderma* species on the growth of *Pythium aphanidermatum* in chilli. *Brazilian Journal of Microbiology*, **42**: 1598-1607.

Nagamma, Gowdra and Nagaraja, A. 2015. Efficacy of biocontrol agents against *Sclerotium rolfsii* causing collar rot disease in chickpea, under *in vitro* conditions. *International Journal of Plant Protection*, **8**(2): 222-227.

Narayana Bhat, M., Sardana, H. R., Dinesh Singh, Chitra Srivastava and Mobin Ahmad. 2015. Evaluvation of chemicals and bioagents against *Sclerotium rolfsii* causing southern blight of bell pepper (*Capsicum annum*). *Indian Phytopathology,* **68**(1): 97-100.

O'Sullivan, D. J. and O'Gara, F. 1992. Traits of *fluorescent pseudomonas* involved in suppression of plant root pathogens. *Microbiological Review,* **56**: 662-676.

Pacheco, K. R., Viscardi, B. S. M., Vasconcelos, T. M. M. D., Morira, G. A. M., Vale, H. M. M. D. and Blum, L. E. D. 2016. Efficacy of *Trichoderma asperellum, Trichoderma harzianum, Trichoderma longibrachiatum* and *Trichoderma reesei* against *Sclerotium rolfsii*. *Bioscience Journal*, **32**(2): 412-421.

Pal, K. K., Dey, R., Bhat, D. M. and Chauhan, S. M. 2000. Enhancement of groundnut growth

yield by plant growth promoting rhizobacteria. *International Archis Newsletter,* **19**: 51-53.

Paramasivam, M. Thaveedu, S. Jhonson, I. and Karthikeyan, M. 2019. Screening of rhizosphere and phylloplane bacterial antagonist against *Sclerotium rolfsii (Sacc.)* in Tropical sugar beet ecosystems. JETIR, **6**(6): 947-952.

Patel, M. I. and Rakholiya, K. B. 2016. *In vitro* evaluation of bio-control agents against *Sclerotium rolfsii* Sacc. *Advance in Life Science.* **5**(4): 1528-1531.

Punja, Z. 1985. The biology, ecology and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology,* **23**: 97-127.

Punja, Z. K. and Damini, A. 1996. Comparative growth, morphology and physiology of three *Sclerotium* species. *Mycologia,* **88**: 694-706.

Quecine, M. C., Araujo, W. L., Marcon. J., Gai, C. S., Azevedo, J. L. and Pizzirani-Kleiner, A. A. 2008. Chitinolytic activity of endophytic *streptomyces* and potential for biocontrol. *Letters in Applied Microbiology,* **47**: 486-491.

Rahel Ratnakumari, Y. R., Nagamani, A. and Bhramaramba, S. 2011. Non-volatile and volatile metabolites of antagonistic *Trichoderma* against collar rot pathogen of *Mentha arvensis. International Journal of Pharmacology and Biomedical Research,* **2**(2): 56-58.

Rahman, M. A., Mahmud, T. M. M., Kadir, J., Rahman, R. A. and Begum, M. M. 2007. Screening of antagonistic bacteria for biocontrol activities on *Colletotrichum gloeosporioides* in papaya. *Asian Journal of Plant Science,* **6**(1): 12-20.

Ramanathan. A. 1989.Bio-control of damping-off disease of chilli due to *Pythium aphanidematum* (Edson) Fitz. *M.Sc. (Ag.) Thesis,* Tamil Nadu Agricultural University, Coimbatore, India.

Ronakkumar, T. V. and Sumanbhai, C. M. 2014. First report of stem rot on cluster bean in Gujarat. *Trends in Bioscience,* **7**: 23.

Sababandy, Faheem amin, Razolan, V. K., Mohiddin, F. A. and Bhat, K. A. 2008. Potential of *Trichoderma* Species as biocontrol agents of Soil borne fungal Propagules. *Journal of Phytopathology,* **2**: 38-41.

Sarma, B. K., Singh, D. P., Singh, H. B., Singh A. and Singh, U. P. 2000. *Sclerotium rolfsii* a threat to crop plants. *Indian Journal of Plant Pathology*, **20**: 1-14.

Sarma, B. K., Singh, U. P. and Singh, K. P. 2002. Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia*, **94**: 1051-1058.

Shiva Kant Kushwaha, Sanjeev Kumar and Balkishan Chaudhary. 2018. Efficacy of *Trichoderma* against *Sclerotium rolfsii* causing collar rot disease of lentil under *in vitro* conditions. *Journal of Applied and Natural Sciences*, **10**(1): 307-312.

Siddique, M. N. A., Abu Ahmmed, A. N. F., Md. Golam Hasan Mazumder, M. O., Khaiyam and Md. Rafiqul Islam. 2016. Evaluation of some fungicides and bio-agents against *Sclerotium rolfsii* and foot and root rot disease of Eggplant (*Solanum melongena* L.). *The Agriculturists*, **14**(1): 92-97.

Singh, N. P., Choudhary, A. K. and Chaudhary, S. P. S. 2001. Variability and correlation studies in some genotypes of cluster bean. *Advances in Arid Legume Research*, **2**(1): 14-18.

Son, J. S., Sumayo, M., Hwang, Y., Kim, B. S. and Ghim, S. 2014. Screening of plant growth-promoting rhizobacteria as elicitor of systemic resistance against gray leaf spot disease in pepper. *Applied and Soil Ecology,* **73**: 1-8.

Sundaramoorthy, S. and Balabaskar, P. 2013. Evaluation of combined efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* in managing tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol). *Journal of Plant Pathology,* **12**: 154-161.

Vincent, J. M. 1929. Distribution of fungal hyphae in the presence of certain inhibitors. *Nature,* **159**: 850.

Watanabe, T. 2002. *Sclerotium* sp. morphologies of cultured fungi and key species: Pictorial Atlas of Soil and Seed fungi. 2nd Edn., CRC Press, New York.

Weller, D. M. 1988. Biological control of soil-borne pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology*, **26**:279-407.

Muthukumar, A[1*]., Suthin Raj, T[2]., Sudha, A[3] and Udayakumar, R[2]

[1&2]Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar-608 002, Chidambaram, Tamil Nadu, India.

[3]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

[*]Corresponding author
e-mail:muthuragulajay@gmail.com